

What is claimed:

1. A method of making a mammalian nuclear transfer embryo that is comprised of cells that are incapable of differentiating into a particular cell lineage, comprising:
  - (a) isolating a differentiated mammalian cell to be used as a nuclear transfer donor;
  - (b) genetically engineering said cell to be incapable of differentiating into a particular cell lineage;
  - (c) effecting nuclear transfer of said differentiated, genetically engineered cell, nucleus or chromosomal DNA thereof into a suitable recipient cell;

thereby forming a nuclear transfer embryo comprised of cells that are incapable of differentiating into a particular cell lineage.

2. The method of claim 1, wherein said nuclear transfer embryo is permitted to develop into a blastocyst or morula.
3. The method of claim 2, wherein said blastocyst, morula or cells derived therefrom are permitted to differentiate.
4. The method of claim 1, wherein said differentiated mammalian cell is a human cell.
5. The method of claim 1, wherein said particular cell lineage into which said nuclear transfer embryo is incapable of differentiating is selected

from the group consisting of endoderm, mesoderm and ectoderm lineages.

6. The method of claim 5, wherein said particular lineage is more specifically selected from the group consisting of cardiomyocytes, hematopoietic stem cells, endothelial cells, pancreatic islet cells, neurons, fibroblasts and keratinocytes, and chondrocytes.
7. The method of claim 5, wherein said differentiated mammalian cell is genetically engineered by knocking out a gene required for differentiation into said particular lineage.
8. The method of claim 1, wherein said differentiated mammalian cell is genetically engineered by stably transfecting said cell with a suicide gene operably linked to a lineage specific promoter expressed during said particular stage of development.
9. The method of claim 5, wherein said differentiated mammalian cell is genetically engineered by stably transfecting said cell with at least one oligonucleotide operably linked to a promoter, wherein said at least one oligonucleotide encodes an RNA molecule that inhibits or interferes with the expression of at least one gene expressed in said particular lineage.
10. The method of claim 9, wherein said interfering or inhibitory RNA molecule is selected from the group consisting of antisense RNAs,

ribozymes and RNA molecules that mediate RNA interference (RNAi) of a target gene or gene transcript.

11. The method of claim 10, wherein said RNA molecule is an antisense RNA that is about 10 to 20 nucleotides or greater in length.
12. The method of claim 10, wherein said RNA molecule is an antisense RNA, and is at least about 75% complementary to its target gene or gene transcript.
13. The method of claim 10, wherein said RNA molecule is a ribozyme selected from the group consisting of hammerhead ribozymes, axehead ribozymes, newt satellite ribozymes, Tetrahymena ribozymes and Rnase P.
14. The method of claim 10, wherein said RNA molecule mediates RNAi of a target gene, and is at least about 100 nucleotides in length.
15. The method of claim 14, wherein said differentiated mammalian cell is genetically engineered with a second RNA molecule that mediates RNAi and is also expressed from an oligonucleotide operably linked to a promoter, wherein said second RNA molecule forms a double stranded RNA with said first RNA molecule following expression, thereby effecting RNAi against the target gene or gene transcript.

16. The method of claim 15, wherein said first and second RNA molecules are expressed from the same gene construct operably linked on either end to convergent promoters such that each promoter directs transcription of the opposite strand of the gene.
17. The method of claim 10, wherein said RNA molecule mediates RNAi of a target gene, and forms a stem-loop or hairpin structure.
18. The method of claim 17, wherein said RNA molecule is at least about 200 nucleotides in length.
19. The method of claim 9, wherein said promoter is lineage specific in that it is only expressed during said particular development lineage.
20. The method of claim 19, wherein said promoter is inducible.
21. The method of claim 1, wherein said suitable recipient cell is a mammalian oocyte or ES cell selected from the group consisting of human, primate, bovine, porcine, sheep, goat, rat, mouse, hamster, guinea pig, horse, birds, amphibians and fish.
22. The method of claim 1, wherein the cells derived from said blastocyst or morula are inner cell mass cells.

23. The cell lineage deficient nuclear transfer embryo made by the method of claim 1.
24. Cell lineage deficient embryonic stem cells derived from the inner cell mass cells of claim 22.
25. The method of claim 7, wherein said particular lineage is the endoderm lineage, and said knockout affects a gene selected from the group consisting of GATA-4 and GATA-6.
26. The method of claim 7, wherein said particular lineage is the mesoderm lineage, and said knockout affects a gene selected from the group consisting of SRF, MESP-1, HNF-4, beta-1 integrin and MSD.
27. The method of claim 7, wherein said particular lineage is the ectoderm lineage, and said knockout affects a gene selected from the group consisting of RNA helicase A and H beta 58.
28. The method of claim 9, wherein said particular lineage is the endoderm lineage, and said at least one gene is selected from the group consisting of GATA-4 and GATA-6.
29. The method of claim 9, wherein said particular lineage is the mesoderm lineage, and said at least one gene is selected from the group consisting of SRF, MESP-1, HNF-4, beta-1 integrin and MSD.

30. The method of claim 9, wherein said particular lineage is the ectoderm lineage, and said at least one gene is selected from the group consisting of RNA helicase A and H beta 58.
31. A human somatic or embryonic cell comprising a heterologous DNA construct or constructs, wherein expression of said heterologous DNA construct or constructs results in a double-stranded RNA molecule that mediates RNA interference (RNAi) of a target gene expressed during embryonic development.
32. The human somatic or embryonic cell of claim 31, wherein said target gene is expressed during a particular cell lineage selected from the group consisting of endoderm, mesoderm and ectoderm.
33. The human somatic or embryonic cell of claim 31, wherein said heterologous DNA construct or constructs are expressed from a lineage specific promoter or promoters.
34. The human somatic or embryonic cell of claim 31, wherein said heterologous DNA construct and constructs are expressed from an inducible promoter or promoters.
35. The human somatic or embryonic cell of claim 31, wherein said double stranded RNA molecule results from hairpin annealing of a single RNA transcript.

36. The human somatic or embryonic cell of claim 31, wherein said double stranded RNA molecule results from annealing of two separate RNA transcripts.
37. A method of making a nuclear transfer embryo comprising cells that are incapable of differentiating into a particular cell lineage, comprising:
- (a) isolating a differentiated mammalian cell to be used as a nuclear transfer donor;
  - (b) stably transfecting into said cell one or more nucleic acid constructs that result in or mediate RNA interference (RNAi) of a target gene expressed in said particular cell lineage;
  - (c) effecting nuclear transfer of said differentiated, genetically engineered cell, nucleus or chromosomal DNA therefrom into a suitable recipient cell;
- thereby forming a nuclear transfer embryo comprising cells that are incapable of differentiating into said particular cell lineage.
38. The method of claim 37, wherein said double stranded RNA molecule is formed via hairpin or stem-loop formation from a single RNA transcript.
39. The method of claim 37, wherein said double stranded RNA molecule is formed by the annealing of separate RNA transcripts.

40. The method of claim 39, wherein said separate RNA transcripts are expressed from the same double stranded DNA construct that is flanked by convergent promoters.
41. The method of claim 37, wherein said differentiated mammalian cell is a human cell.
42. The method of claim 41, wherein said suitable recipient cell is a mammalian oocyte or ES cell selected from the group consisting of human, primate, bovine, porcine, sheep, goat, rat, mouse, hamster, guinea pig, horse, birds, amphibians and fish.
43. The method of claim 37, wherein said nuclear transfer embryo is incapable of differentiating into a cell lineage selected from the group consisting of endoderm, mesoderm and ectoderm.
44. The transfected differentiated mammalian cell formed in step (b) of claim 37.
45. The cell lineage deficient nuclear transfer embryo made by the method of claim 37.
46. The human cell lineage deficient nuclear transfer embryo made by the method of claim 41.



47. The method of claim 37 further comprising permitting said nuclear transfer embryo to develop into a morula or blastocyst.
48. The method of claim 47, wherein said blastocyst, morula or cells derived therefrom are permitted to differentiate.
49. The method of claim 48, wherein the cells derived from said morula or blastocyst are inner cell mass cells.
50. Cell lineage deficient embryonic stem cells derived from the inner cell mass cells of claim 49.
51. Differentiated cells made by the method of claim 3.
52. A tissue engineered using the differentiated mammalian cells of claim 51.
53. Differentiated cells made by the method of claim 48.
54. A tissue engineered using the differentiated human cells of claim 53.
55. The method of claim 1, further comprising a step between steps (b) and (c) wherein said differentiated mammalian donor cell is further

genetically engineered by deleting or modifying at least one harmful or undesirable DNA or by inserting at least one therapeutic or corrective DNA.

56. The method of claim 37, further comprising a step between steps (b) and (c) wherein said differentiated mammalian donor cell is further genetically engineered by deleting or modifying at least one harmful or undesirable DNA or by inserting at least one therapeutic or corrective DNA.
57. The method of claim 41, further comprising a step between steps (b) and (c) wherein said differentiated human donor cell is further genetically engineered by deleting or modifying at least one harmful or undesirable DNA or by inserting at least one therapeutic or corrective DNA.
58. The genetically modified human nuclear transfer embryo isolated by the method of claim 56.
59. The genetically modified nuclear transfer embryo isolated by the method of claim 57.
60. A method of isolating genetically modified differentiated human cells of a desired lineage comprising growing the nuclear transfer embryo of claim 58 in such a manner as to permit differentiation into a desired lineage.

61. The genetically modified differentiated human cells of a desired lineage isolated by the method of claim 60.
62. A tissue engineered using the genetically modified differentiated human cells of claim 61.
63. A method of therapy using the differentiated mammalian cells of a desired lineage of claim 51.
64. A method of therapy using the differentiated mammalian cells of a desired lineage of claim 53.
65. A method of therapy using the genetically modified differentiated human cells of a desired lineage of claim 61.
66. A method of transplantation using the engineered mammalian tissue of claim 52.
67. A method of transplantation using the engineered human tissue of claim 54.
68. A method of transplantation using the genetically modified engineered human tissue of claim 62.

69. The method of claim 1, wherein said differentiated mammalian cell nuclear transfer donor is either a somatic cell or an embryonic cell.
70. The method of claim 37, wherein said differentiated mammalian cell nuclear transfer donor is either a somatic cell or an embryonic cell.
71. The method of claim 37, wherein said one or more nucleic acid constructs that result in or mediate RNAi include DNA constructs that upon expression result in the formation of a double stranded molecule, and single stranded or double stranded RNA molecules.
72. The method of claim 71, wherein said DNA constructs either integrate into the chromosome or are expressed episomally.